nature portfolio

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	eSerialEM 4-0-9
Data analysis	relion 3.0.8, UCSF Chimera 1.15, PyMoL 4.6.0, Clustal X 1.3, ESPript 3.0, Coot 0.9.2, PHENIX 1.18.2

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The cryo-EM maps and coordinates have been deposited to the Electron Microscopy Data Bank (EMDB) and Protein Data Bank (PDB). The transcribing complexes include ASFV RNA polymerase core (EMD-39507, PDB ID 8YQV), ASFV RNA polymerase-M1249L complex1 (EMD-39506, PDB ID 8YQU), ASFV RNA polymerase-M1249L complex2 (EMD-39505, PDB ID 8YQT), ASFV RNA polymerase-M1249L complex3 (EMD-39508, PDB ID 8YQW), ASFV RNA polymerase-M1249L complex4 (EMD-39509, PDB ID 8YQX), ASFV RNA polymerase-M1249L complex complete (EMD-39510, PDB ID 8YQY), ASFV RNA polymerase-M1249L-DNA complex

(EMD-39511, PDB ID 8YQZ), ASFV RNA polymerase-M1249L complex5 (EMD-39514), ASFV RNA polymerase-M1249L complex6 (EMD-39512), ASFV RNA polymerase-M1249L complex7 (EMD-39513), ASFV RNA polymerase-M1249L complex8 (EMD-39516), ASFV RNA polymerase-M1249L complex9 (EMD-39515), ASFV RNA polymerase-M1249L complex10 (EMD-39517), ASFV RNA polymerase-M1249L complex11 (EMD-39518), ASFV RNA polymerase-M1249L complex12 (EMD-39519), ASFV RNA polymerase-M1249L complex13 (EMD-39521), ASFV RNA polymerase-M1249L complex14 (EMD-39520), ASFV RNA polymerase-M1249L complex15 (EMD-39522), ASFV RNA polymerase-M1249L complex16 (EMD-39523), ASFV RNA polymerase-M1249L complex17 (EMD-39524), ASFV RNA polymerase-M1249L complex18 (EMD-39525), ASFV RNA polymerase-M1249L complex19 (EMD-39526), ASFV RNA polymerase-M1249L complex20 (EMD-39527), ASFV RNA polymerase-M1249L complex21 (EMD-39528), ASFV RNA polymerase-M1249L complex22 (EMD-39530), ASFV RNA polymerase-M1249L complex23 (EMD-39531), ASFV RNA polymerase-M1249L complex24 (EMD-39529), and ASFV RNA polymerase-M1249L complex25 (EMD-39536). The raw sequencing data reported in this paper were submitted to Genome Sequence Archive (GSA) of National Genomics Data Center (http://bigd.big.ac.cn, Bioproject number: PRJCA003613).

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	the Ministry of Agriculture and Rural Affairs

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For the cryo-EM analysis, the number of micrographys is determined by the available microscope time. The sample size of cell-based assays was performed three times, which is sufficient for statistic analysis.
Data exclusions	No data were excluded from analyses.
Replication	Sample preparation-related experiments including protein purification and enzymatic assays were reproduced at least three times independently. All attempts at replication were successful.
Randomization	Randomization is not relevant to cryo-EM and other experiments, because the sample were not allocated into experimental groups during data acqyisition and analysis.
Blinding	Blinding is not relevant to this study. The parameters for biochemistry, cryo-EM, and any other experiments in this study did not require subjective assessments of the treatments.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Anti-mouse antibodies used in WB :1. WB Anti-Strep tag Monoclonal Antibody (Solarbio, Clone 7A10, 1□5000).
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Porcine alveolar macrophages (PAMs) and bone marrow cells (PBMs) were isolated from 5-week-old healthy specific pathogen free (SPF) piglets.
Authentication	Cell line used in the studies is freshly prepared and cultured. No authentication procedure is needed. authenticated.
Mycoplasma contamination	We have confirmed that cell lines are tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Porcine alveolar macrophages (PAMs) and bone marrow cells (PBMs) were isolated from 5-week-old healthy specific pathogen free (SPF) piglets.
Wild animals	No wild animals is involved in our study.
Reporting on sex	Sex is not considered in the study design.
Field-collected samples	Not relevant to our study.
Ethics oversight	Harbin Veterinary Research Institute (HVRI) of the Chinese Academy of Agricultural Sciences (CAAS).

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*